Salidroside Decreases Atherosclerotic Plaque Formation in Low-Density Lipoprotein Receptor-Deficient Mice

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Salidroside is isolated from Rhodiola rosea and is one of the main active components in Rhodiola species. The present study was designed to evaluate the effects of Salidroside on atherosclerotic plaque formation in high-fat diet-(HFD-) fed female LDL receptor knockout (LDLr−/−) mice. LDLr−/− mice fed an atherogenic HFD for 12 weeks were divided into two groups. One group was administered Salidroside (50 mg/kg/oral gavage) daily for 8 weeks, while the control group was administered saline. Salidroside treatment reduced serum lipids levels and the plaque area through the arch to the abdominal aorta. Furthermore, Salidroside improved macrophage content and enhanced collagen and smooth muscle cells contents in the aortic sinus. These changes were associated with reduced MCP-1, VCAM-1, and VCAM-1 protein expression in atherosclerotic aortas. All these results suggest that Salidroside decreases atherosclerotic plaques formation via effects on lipid lowering and anti-inflammation in HFD-fed LDLr−/− mice.

1. Introduction

Atherosclerosis (AS) is a widespread and one of the most dangerous cardiovascular diseases which cause considerable threat to human health worldwide. Accordingly, treatment aimed at AS is of great clinical importance. However, an ideal drug against atherosclerosis is still lacking [1]. In China, drugs of herbal origin with low side effects are of high interest as alternative therapy, and medicinal plants may have potential to stabilize atherosclerotic plaques [2].

Rhodiola rosea has long been used as a medicinal plant and has been reported to have various pharmacological properties, including antifatigue and antistress activity [3], anticancer, antioxidant and immune enhancing and stimulating sexual activity [4], anti-inflammation [5], improvement of glucose and lipid metabolism [6, 7], antiarrhythmic effect [8], and enhancement of angiogenesis [9]. However, the effects of Rhodiola rosea on atherosclerotic lesions formation are still unclear. The aim of the present study was to evaluate the effects of Salidroside (p-hydroxyphenethyl-β-d-glucoside, one of the main active components in Rhodiola species) on atherosclerotic plaque formation in high-fat diet-(HFD) fed female LDL receptor knockout (LDLr−/−) mice.

2. Materials and Methods

2.1. Animal Model. Female LDLr−/− mice (C57BL/6 genetic background) were obtained from the Jackson Laboratory (Bar Harbor, ME, USA). A total of 30 LDLr−/− mice (3 weeks old, weight 19 to 21 g) were used in the study. All animal procedures were performed in compliance with “The Guide for the Care of Use of Laboratory Animals” published by the National Institute of Health (NIH Publication No. 85-23, revised 1996) and approved by the Animal Care Committee of Tongji University School of Medicine. Mice were fed a high-fat diet (containing 18% hydrogenated cocoa butter, 0.15% cholesterol, 7% casein, 7% sucrose, and 3% maltodextrin) for 12 weeks, starting at 4 weeks of age. Thirty mice were further divided into two groups randomly (N = 15 per group). One group received Salidroside (≥98% purity, purchased from Yuanye Technology Co., Ltd., Shanghai,...
China) 50 mg/kg/oral gavage once daily. The Salidroside dose
used was selected from a pilot experiment, in which we aimed
at only 20% cholesterol reduction to ensure atherosclerosis
development within a reasonable time period, while the
other group received saline. During the 8 weeks of treatment,
all mice allowed free access to a high-fat diet and water.

2.2. Serum Lipid Analysis. Blood samples were taken after a
4-hour fast by tail bleeding throughout the study. Serums
were acquired through centrifugation of the blood samples
at 4°C at 1000 g and stored at −80°C until analysis. Total
cholesterol (TC), high-density lipoprotein cholesterol (HDL-
C), and triglyceride (TG) levels were measured enzymatically
with commercial kits from Wako Inc. (Richmond, USA)
using an auto-analyzer (Hitachi 7100, Tokyo, Japan).

2.3. Morphology of Atherosclerotic Plaques. After 8 weeks
of treatment, mice were anesthetized by diethyl ether
and sacrificed. The mice were dissected and aortas were
perfusion-fixed with 4.5% formaldehyde. Then the aortas
were dissected from the heart to approximately 3 mm distal
to the iliac bifurcation. The aortas were preserved in fresh
paraformaldehyde solution for 2 weeks, and oil red O
staining was employed to determine the plaques on entire
aortas. Briefly, after removing surrounding adventitial fatty
tissue, the aortas were opened longitudinally and pinned
out on a black silica gel plate. The aorta was rinsed in 70%
acetone/35% ethanol for about 10 minutes, and washed in
80% ethanol for 5 minutes. For collagen determination,
picric and sirius staining was performed, according to the
manufacturer’s instructions (Genmed Inc., Arlington, USA).
Finally, the stained aortas were photographed and analyzed
using NIH Image Pro-Plus 6.0 software (NIH, Bethesda,
MD).

2.4. Immunohistochemical Staining of α-SMA and MAC-3 in
the Aortic Sinus. Isolated aortic sinus tissues were fixed by
immersion in 4% paraformaldehyde for 48 h at 4°C and
incubated with 30% sucrose for 2 days. Each aortic sinus
was embedded in paraffin. The paraffin-embedded sections
(5 μm thick) were placed on poly-L-lysine-coated slides.
The slides were air dried overnight at room temperature,
wrapped, and stored at −70°C until immunostaining. Slides
were immersed in 0.3% H2O2 for 10 minutes to abolish
endogenous peroxidase activity and rinsed with PBS. And
then, slides were incubated with 5% BSA for 1 hour at room
temperature to block nonspecific staining and incubated
with a primary antibodies of murine α-smooth muscle
actin (α-SMA) antibody (1 : 50 dilution; Santa Cruz Inc.,
California, USA), MAC-3 (1 : 50 dilution; Beyotime Biotech
Inc., Jiangsu, China) in humidified chambers for overnight
at 4°C. All slides were incubated with biotinylated secondary
antibody for 1 hour at room temperature and then incubated
with horseradish peroxidase-conjugated streptavidin
for 20 min at room temperature, followed by detection with
a DAB kit (Beyotime Biotech Inc., Jiangsu, China). For the
quantitative analysis, the average score of 10–20 randomly
selected area was calculated using NIH Image Pro-Plus 6.0
software.

2.5. Western Blot Analysis. Aorta sinus tissues were snap-
frozen in liquid nitrogen, pulverized, and resuspended
in ice-cold lysis buffer (Solarbio). Protein concentrations
were determined with the Bradford method. Lysates were
allowed to solubilize on ice for 30 min, and particulate
mass was removed by centrifugation (15,000 g) for 15 min
at 4°C. Supernatants were analyzed by SDS-PAGE. Primary
antibodies used included intercellular adhesion molecule 1
(ICAM-1, 1 : 1000 dilution), vascular cell adhesion molecule
1 (VCAM-1, 1 : 1000 dilution) and monocyte chemotactic
protein-1 (MCP-1, 1 : 500 dilution) were purchased from
Santa Cruz Inc. (California, USA). Secondary antibodies
were horseradish peroxidase-labeled antibodies (Thermo
Scientific Pierce, Rockford, USA). Blots were processed for
enhanced chemiluminescence using a Pierce ECL Western
blotting substrate (Thermo Scientific Pierce, Rockford,
USA).

2.6. Statistical Analysis. All statistical analyses were carried
out with GraphPad PRISM 5.0 statistical software (San
Diego, California, USA). Quantitative variables are expressed
as means ± SD. Two-tailed Student’s t-tests were used to
compare continuous data for between-group differences. P <
0.05 was considered statistically significant.

3. Results

3.1. Body Weights and Biochemical Studies. The weight of
mice was 20.09 ± 0.40 g at baseline but increased to
31.68 ± 0.15 g at week 24 (P < 0.001). However, there
was no significant difference in body weight among the
two groups at either baseline or week 24. Compared with
the vehicle group receiving no drugs for 8 weeks, mice
received Salidroside showed lower levels of TC and TG
and significantly increased HDL-C (Table 1). These findings
suggest that Salidroside intervention may help to restore the
lipid imbalance induced by HFD.

3.2. Salidroside Significantly Reduces the Formation of
Atherosclerotic Lesions. To ascertain the effects of Salidroside
on atherosclerotic lesion formation, we detected plaque sizes
at the aorta via oil red O staining. As shown in Figure 1,
Salidroside induced a significant decrease in the plaque
area. Vehicle-treated mice group displayed approximately
69.29 ± 0.04% plaque coverage, whereas mice treated with
Salidroside had 32.71 ± 0.02% plaque coverage.

To evaluate lesion composition, we immunostained
the lesions for macrophages, vascular smooth muscle cells
(VSMCs), as well as collagen content. The positive staining
area of α-actin in the Salidroside-treated groups was signifi-
cantly higher than that in the vehicle group (6.21 ± 1.48% versus
3.25 ± 1.17%, P < 0.01). Similarly, the area positively
stained with sirius red in the Salidroside-treated groups was
significantly higher than that in the vehicle group (14.51 ±
Table 1: Serum lipid profile in two groups after 8-week treatment.

<table>
<thead>
<tr>
<th>Groups (n = 15)</th>
<th>TC (mg/dL)</th>
<th>TG (mg/dL)</th>
<th>HDL-C (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>1178.7 ± 123</td>
<td>463.6 ± 33</td>
<td>18.5 ± 2.1</td>
</tr>
<tr>
<td>Salidroside</td>
<td>524.3 ± 65***</td>
<td>237.5 ± 17***</td>
<td>20.7 ± 1.6**</td>
</tr>
</tbody>
</table>

TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein. ***P < 0.001 **P < 0.01 versus vehicle group.

3.3. Salidroside Decreases ICAM-1, VCAM-1, and MCP-1 Expression in the Lesion Areas. We additionally evaluated the expression of several inflammatory mediators in the aortic arch plaques using Western blot. As shown in Figure 3, the protein levels of ICAM-1, VCAM-1, and MCP-1 in the Salidroside-treated groups were significantly lower than that in the vehicle group (all P < 0.01).

4. Discussion

The aim of this study was to evaluate the effects of Rhodiola rosea on atherosclerosis development in high-fat diet-fed mice. Salidroside has been shown to reduce atherosclerotic plaque formation, serum levels of lipids, and vascular inflammatory markers. These results demonstrated for the first time that Rhodiola rosea provides a novel approach to against atherosclerosis.
Evidence-Based Complementary and Alternative Medicine

Vehicle Salidroside

ICAM-1

VCAM-1

MCP-1

GAPDH

(a)

Vehicle

Salidroside

(b)

Figure 3: Salidroside attenuates inflammatory mediators expression at the aortic arch. (a) Representative western blotting for ICAM-1, VCAM-1, and MCP-1. (b) Quantitative analysis (N = 5 per group), **P < 0.01 versus vehicle group.

Previous studies have demonstrated that atherosclerosis is a complex and perpetuating inflammatory disease involving the aorta and its major branches, and the distinctive histological features of vulnerable plaques in humans include a large lipid core, a thin fibrous cap depleted of extracellular matrix and VSMCs, active inflammation, outward or positive remodeling, and increased adventitial and plaque neovascularity [10]. In the present study, Salidroside treatment decreased protein levels of ICAM-1, VCAM-1, and MCP-1 in plaques. In addition, the macrophage content in the lesion area in the Salidroside-treated group was lower than that of the vehicle group. All of these inflammatory factors were previously shown to be involved in inflammatory cascade and have been implicated in the pathogenesis of atherosclerosis and plaque destabilization [11].

Another noteworthy finding is that the collagen and VSMCs contents in the lesion area in the Salidroside-treated group were higher than that of the vehicle group. Vascular remodeling, especially extracellular matrix (ECM) increase, is thought to stabilize plaques, which may prevent disruption of lesions [12]. Based on the present data, we propose that Salidroside increases the collagen content or decreases degradation of collagen in the lesion areas, indicating that Salidroside stabilizes plaques.

Our study contains several limitations. First, although components of Salidroside were clear, the dose-effect relationship of Salidroside on atherosclerotic plaques progression remains to be clarified. Second, although the antiatherosclerosis effects of Salidroside have been confirmed, the detailed molecular mechanism of the lipid-lowering and anti-inflammation effects requires further investigation.

In summary, our in vivo studies demonstrate that Salidroside decreases atherosclerotic plaques formation via effects on lipid lowering and anti-inflammation in high-fat diet-fed LDLr−/− mice. Thus, treatment with Rhodiola rosea provides a new therapeutic approach to prevent atherosclerosis.

Conflict of Interests

The authors have no conflict of interests.

Authors’ Contribution

B. C. Zhang and W. M. Li contributed equally to this work.

Acknowledgments

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References


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